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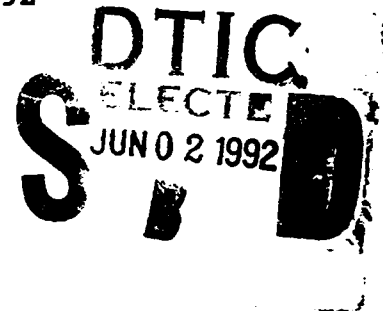
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TITLE: RETROVIRUS STUDIES IN NONHUMAN PRIMATES AT FOUR REGIONAL
PRIMATE RESEARCH CENTERS

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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Interim Report

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9. Comments for Administrative and Logistical Matters

For the CRPRC:

None

For the DRPRC:

None

For the NERPRC:

None

For the YRPRC:

None

10. Scientific Progress

For the CRPRC:

SPECIFIC AIM 1.1: In collaboration with Dr. Paul Luciw, recombinant envelope glycoprotein, gp 120 of SIV has been expressed and produced in Chinese hamster ovary cells. The glycoprotein has been purified and immunization of rhesus macaques with purified CHO expressed gp 120 is presently in progress.

SPECIFIC AIM 1.2: To determine if actively and passively acquired SIV/SM specific antibody protects against a cell-associated challenge. These antibodies produced by psoralen-UV inactivated virus immunization, although they had neutralizing capacity failed to protect animals in an passive immunization experiment. In fact, there is suggestive evidence that antibodies accelerated the clinical disease. These experiments thus suggest that enhancement may be in vivo as well as an in vitro phenomenon. There were 4 control animals with virus alone and 4 with passive inunoglobin.

SPECIFIC AIM 1.3: To test for SIV transmission and seroconversion following inoculation of the vaginal mucosa with SIV-infected splenocytes. The Heterosexual Transmission of AIDS: A Simian Model. The overall objective of this project is to establish an animal model for the heterosexual transmission of AIDS. The model system is simian immunodeficiency virus (SIV) infection of the rhesus macaque. SIV, a lentivirus is closely related to HIV and produces an immunodeficiency syndrome in Asian macaques that is very similar to human AIDS. Two strains of SIV have been used in this study, the SIV isolate from rhesus macaques which is designated SIVMAC and an SIV isolate from the sooty mangabey monkey which is called SIVSM.

Progress to Date: To establish a model for the sexual transmission of AIDS, it was necessary to first show that simian immunodeficiency virus (SIV) could be transmitted across the genital mucosa of males and females.

Genital transmission of SIV to female rhesus macaques. We have previously reported that SIV can be transmitted across the vaginal mucosa of female rhesus macaques (Miller, et. al., 1990). To determine the effect of virus dose on the genital transmission of SIV in female rhesus macaques, cell-free SIVmac (Miller, et., al., 1990) was infused through a 2.5mm outer diameter (8 French) soft, plastic, pediatric nasogastric feeding tube (American Pharmaseal, Valencia, Ca.) into the vaginal vault of female rhesus macaques immobilized with Ketamine HCl. After each inoculation, the animals remained immobile for 15 minutes.

To determine the infectious dose of cell-free virus required for the genital transmission of SIV and to determine if cell-free virus, in the absence of co-factors, was capable of producing infection; 4 mature female rhesus macaques were inoculated with a cell-free stock of SIVmac. Two animals received 1 ml of undiluted SIVmac virus stock (50 TCID50)

and 2 animals received 1 ml of a 10-fold dilution. The inoculum was infused into the vaginal vault of the animals 8 times (twice a week for 4 weeks). SIV was isolated from the PBMC of the 2 females inoculated with the undiluted cell-free SIV by day 14 (after 4 inoculations). Of the 2 females that received the 10-fold dilution of virus, one animal was positive on day 14 (after 4 inoculations) and the other is virus negative at 20 months post-inoculation (PI). Western blot analysis of serum from these 4 animals revealed that the 3 females that became viremic were seropositive for SIV specific antibodies by 6 weeks PI while the one uninfected female has not seroconverted by 14 months PI. Of the 3 infected females, 2 are clinically normal and viremic at twenty months PI; the third was euthanized due to severe anemia and thrombocytopenia at 6 months PI.

In addition to the above group of adults, 4 juvenile female rhesus macaques were vaginally inoculated with undiluted cell-free SIVmac (50 TCID₅₀) twice a week for 2 weeks. All 4 of the animals were virus positive by 14 days PI and all were seropositive by 4 weeks PI. At 12 months PI, two of these animals are healthy, and two animals have been euthanatized due to their poor clinical condition. At necropsy, one animal had interstitial pneumonia and enterocolitis, while the other had hepatitis, peritonitis and pancreatitis.

To determine the minimum dose of SIV needed to infect female rhesus macaques by the vaginal route, 4 females were inoculated with 1 ml of cell-free SIV (5 TCID₅₀) once on day 13 of the menstrual cycle. All 4 females remained virus negative and seronegative for 6 months. After 4 months, the same animals were again inoculated with 1 ml of cell-free SIV (50 TCID₅₀) once on day 13 of the menstrual cycle. Three of the animals became viremic by 14 days PI and these animals were seropositive by 4 weeks PI. One animal is virus negative and seronegative at 4 months PI. All the animals are clinically normal at 12 months PI. In addition, 9 more mature female rhesus have been inoculated with 1 ml of cell-free SIVmac (50 TCID₅₀) and 7 were viremic by 14 days PI. Two animals are virus negative and seronegative at 9 months PI. Thus, 10 of 13 female rhesus vaginally inoculated once with cell-free SIV mac became infected.

Effect of nonoxynol-9 containing spermicide on the genital transmission of SIV in female rhesus macaques. In an attempt to prevent the genital transmission of SIV to female rhesus macaques, 1 ml of contraceptive foam containing nonoxynol-9 (12.5% v/v) was infused into the vaginal canal of 6 animals just prior to the inoculation of 1 ml of cell-free SIV (50 TCID₅₀). This sequence was repeated a total of 4 times over 2 weeks. (This dose of SIV had previously produced infection in 4 of 4 females.) Three of 6 animals were virus positive at 2 weeks PI and seropositive at 4 weeks PI. The other 3 animals are virus negative and seronegative at 15 months PI. Thus, nonoxynol-9 containing spermicides provide partial protection against the genital transmission of SIV.

Genital transmission of SIV to male rhesus macaques. To transmit SIV to male rhesus macaques by the genital route, cell-free SIVmac was gently

infused onto the urethral mucosa of immobilized males by inserting a 2.5mm outer diameter (8 French) soft, plastic, pediatric nasogastric feeding tube (American Pharmaseal, Valencia, Ca.) approximately 1 cm into the urethra. Although the animals remained immobile for 15 minutes, some of the inoculum drained from the urethra and came into contact with skin surrounding the urethral orifice. To infect males with SIV, two mature male rhesus macaques were inoculated with 1 ml of cell-free SIVmac (25 TCID50) once a week for three weeks. Both animals became viremic by 31 days PI. One male had a strong antibody response to SIV antigens by 6 weeks PI. This animal developed lymphadenopathy and splenomegaly but was alive and viremic at 30 months post-inoculation. In contrast, the other male developed only a weak antibody response and at 6 months PI became moribund with persistent diarrhea and severe weight loss. At necropsy, this animal had disseminated cytomegalovirus infection, ulcerative gastritis and severe interstitial pneumonia with multinucleate giant cells.

In addition to the above group of adult males, 4 juvenile male rhesus macaques were inoculated intraurethrally with cell-free SIVmac (50 TCID50) twice a week for 2 weeks. All were viremic by 14 days PI and seropositive by 4 weeks PI. All four animals are healthy at 10 months PI.

To determine if it was possible to transmit SIV to males by placing the virus on the intact skin of the penis, 1 ml of cell-free SIVmac (50 TCID50) was placed on the foreskin and glans of the penis of 4 mature male rhesus macaques. Some of the virus inoculum came in contact with the mucocutaneous junction of the urethral os. This procedure was performed twice in 5 days. Two of these animals were viremic by 14 days PI and seropositive by 4 weeks PI. The 2 others are virus negative and seronegative at 9 months PI.

These results show that male and female rhesus macaques can be infected via the genital mucosa. Furthermore, the disease that eventually developed in one male and 2 females was indistinguishable from that seen in intravenously inoculated animals.

Isolation of SIV from genital secretions. Vaginal secretions from 5 SIV-infected female rhesus macaques were collected three times (once every 2 weeks for 6 weeks) and cultured. To isolate virus from vaginal secretions, the vaginal vault was lavaged with 2 mls of RPMI-1640 (containing 1 mg/ml Amphotericin B) and the wash was collected. Samples contaminated with menstrual blood were discarded. The mononuclear cells in the lavage fluid were separated by gradient centrifugation on Ficoll-Hypaque, washed and cultured for SIV as described previously (Miller, et. al., 1990). The supernatant fluid was collected, filtered through a 0.45 micron filter and cultured for SIV as described (Miller, et. al., 1990). Vaginal secretions from 5 SIV-infected female rhesus macaques were collected three times (once every 2 weeks for 6 weeks) and cultured. Samples contaminated with menstrual blood were discarded. SIV was isolated from the cells and cell-free filtrates from the vaginal secretions of 2 of 5 females on the first collection, from the cells and

filtrate from 1 of 4 animals on the next collection, and from the cells of one female and the cells and filtrates of 2 females on a third collection. The positive samples were collected at all stages of the menstrual cycle and all the animals were healthy and viremic. These findings suggest that SIV can be shed at any stage of the menstrual cycle and the fact that virus was never recovered from the vaginal secretions of 2 animals suggest that individual factors may be important in determining if SIV is present in vaginal secretions.

We have previously reported that SIV can be isolated from the semen of SIV-infected male rhesus macaques (Miller, et. al. 1990).

Significance. The initial objective of this project was to determine if a nonhuman primate model for the sexual transmission of AIDS could be developed in the SIV/rhesus macaque model of AIDS. Our findings show that rhesus monkeys can be infected via the genital tract and that this model can be further developed to test the role of chemical and pharmacologic contraceptives as well as surgical contraceptive procedures in the prevention/enhancement of HIV transmission in humans. This model will have an impact on AIDS research of others in that a practical model for studying the sexual transmission of AIDS is feasible and being developed.

We have defined the dose of cell-free SIV necessary for genital transmission in female rhesus macaques. By using the smallest challenge dose of cell-free SIV that is known to produce viremia in 100% of inoculated animals, we can more precisely assess the ability of spermicides and other agents to prevent the genital transmission of SIV.

We have begun to use the animal model we developed to explore specific strategies to prevent the heterosexual transmission of HIV. We have shown that spermicides containing nonoxynol-9 do provide partial protection against the genital transmission of SIV to female rhesus macaques. Although it has been previously shown that N-9 can inactivate HIV in vitro, this is the first attempt to stop the genital transmission of SIV using N-9.

The finding that SIV can be transmitted to male macaques by placing the virus on the skin of the penis, suggests that it may be easier to transmit HIV to males by heterosexual contact than has been appreciated.

SPECIFIC AIM 1.4: Assessment of the ability of SIV to infect New World monkey species.

This study was completed and results are presented in a separate report.

SPECIFIC AIM 1.5: To put in place a simian retrovirus reference laboratory to screen human and non-human primate sera for antibodies against SIV, STLV, and SRV, and to seek new isolates for captive and wild populations of non-human primates. This laboratory is now well established, having tested over 10,000 non-human primate sera for retroviral antibodies. Approximate overall prevalences for the three

virus groups are as follows. SIV: 0% in macaques, 8-10% in African species. STLV: 10% in macaques, 17% in African species. SRV: 5% in macaques, 0% in African species. The major focus of the laboratory is now to provide serologic testing and virus isolation in support of NIH contract facilities for the development of specific pathogen-free (SPF) rhesus monkey breeding colonies. Activities of the laboratory have led to new SIV isolates from a sooty mangabey in West Africa, and from a captive mandrill.

Publications

Miller C.J., Alexander N.J., Sutjipto S., Lackner A.A., Hendrickx A.G., Gettie A., Lowenstine L.J., Jennings M., and Marx P.A. Genital mucosal transmission of simian immunodeficiency virus: Animal model for heterosexual transmission of human immunodeficiency virus. J. Virology, 63:4277-4284, 1989. (Appendix A)

Marx P.A., Miller C.J., Alexander N.J., Sutjipto, S., Lackner A.A., Gettie A., Jennings M., and Hendrickx A.G. An animal model for sexual transmission of HIV. In Heterosexual Transmission of AIDS. Eds. N. Alexander, H.L. Gablenick, and J.M. Spieler. Alan R. Liss, Inc., 1990.

In Press

Miller C.J., Alexander N.J., Sutjipto S., Joye S.M., Hendrickx A.G., Jennings M., Marx P.A. Effect of virus dose and nonoxynol-9 on the genital transmission of SIV. J. of Medical Primatology. In press.

For the DRPRC:

SPECIFIC AIM 2.1: Serial Sacrifice Studies

We are continuing to study the tissues from all of the sacrificed animals by immunohistochemistry for viral and cell-surface antigens, by electron microscopy, and by *in situ* hybridization. We hope to submit the results of some of these studies for publication by the end of 1990. Preliminary results are available for some tissues.

Mononuclear cell inflammation was observed in the portal areas of the liver of 16 infected monkeys, and was associated with bile duct proliferation in 7. *In situ* hybridization revealed occasional positive mononuclear cells in inflammatory infiltrates and sinusoids. Hepatocytes and biliary epithelium were negative. The biliary changes were similar to those that occur in many AIDS patients, but are apparently not due to direct lentiviral effects. The changes may be caused by mediators of inflammation released by infiltrating inflammatory cells.

Glomerulosclerosis and associated tubular changes occurred in the kidneys of 3 of 4 monkeys sacrificed 24 weeks after inoculation and in 3

of 4 moribund animals. Electron microscopy revealed marked expansion of mesangium, thickening of basement membranes, and swelling of endothelial cells. No dense deposits of viral particles were observed. Immunohistochemistry did not detect viral antigens, IgG, IgM, IgA, complement, or fibrinogen. By *in situ* hybridization, rare glomerular cells were positive. These results suggest that this lesion is not caused by immune complex disease. The glomerular lesion in these monkeys resembles the lesion that occurs in a small percentage of AIDS patients.

The synovial membranes of many animals had changes consisting of focal villous synovial cell hyperplasia and perivascular mononuclear inflammatory cell infiltration. Immunohistochemistry and electron microscopy detected viral proteins and particles in some infiltrating inflammatory cells (syncytial cells) in the synovium. These results indicate that SIV is a primary joint pathogen. Arthralgia is a common symptom in AIDS patients, but little is known concerning its pathogenesis.

Two animals inoculated with SIVBK28 were sacrificed 26 weeks after inoculation. The only change observed at necropsy was lymphoid hyperplasia, although one of the monkeys was antigenemic. The remaining two animals will be followed clinically until they become moribund, when they will be sacrificed. They are currently healthy. The monkeys inoculated in the second year with SIVBK28 were remarkably different from those inoculated in the first year with SIVDeltaB670. Although all became infected, the animals inoculated with SIVBK28 had fewer clinical problems than those inoculated with SIVDeltaB670, and those problems which did occur were less severe. Four animals developed mild rashes, only two of which were the typical "SIV rash" seen within 2 weeks post-inoculation. Although there were several animals with diarrhea, most responded to antibiotic therapy and did not have recurrent episodes. Two cases of diarrhea were caused by *Campylobacter coli* and although *Shigella* was suspected in several animals, based on elevated WBC and the response to antibiotic therapy, no clear cases of shigellosis were diagnosed. *Giardia* was identified in only one animal and was nonresponsive to therapy. Lymphadenopathy occurred rarely in this group. Enlarged lymph nodes were observed in only two animals and this did not occur until greater than 100 days after infection. Splenomegaly did not occur in any of these animals. There were two cases of oral candidiasis. One animal had thrombocytopenia with a decrease in platelets from 435,000 to 43,000/ml where it remained for one month prior to sacrifice. No signs of a bleeding disorder developed in this animal. One interesting observation was made involving the liver enzymes in these animals. All five animals which remained alive beyond 5/09/89 developed transient elevations in SGPT, SGOT, and GGT at 84-114 days post-inoculation, with values increasing 2-4 times the normal value. This may indicate a transient episode of hepatitis in these animals and further testing will be done to confirm this possibility. None has become moribund. Only 4 individuals had SIV core antigen detectable in their serum on a single occasion each, 2 at 2 weeks and 2

at 20 weeks after inoculation. Significant lymphocyte subset changes were limited to 3 individuals that had elevated levels of circulating B-cells. The only significant necropsy finding was lymphoid hyperplasia in most animals. No viral core proteins could be detected in any lymphoid tissue by immunohistochemistry, although rare cells were positive for VRNA by in situ hybridization. These results indicate that the infectious molecular clone SIVBK28 is nonpathogenic in rhesus monkeys, and that the loss of pathogenicity is associated with a marked reduction of viral expression in tissues. Pathogenic strains of SIV may eventually evolve in these animals however.

In November 1989 we published a case report of two animals from this study with adenovirus enteritis. This is the first report of this type of lesion in a nonhuman primate.

Publications Supported in Part by Army Funds:

Baskin GB, Soike KF. Adenovirus enteritis in SIV-infected rhesus monkeys. J Inf Dis 160:905-907, 1989.

Baskin GB, Jeubler D, Hu F-S, Murphey-Corb M., Martin LN, Fairburn BD. Distribution of SIV in serially sacrificed rhesus monkeys. Proceedings of the Symposium on Nonhuman Primate Models for AIDS, 1989, Abstract #84.

Martin L, Murphey-Corb M, Baskin G, Davison-Fairburn, B. Predictive indicators for experimental SIV-induced disease progression in young rhesus monkeys. Proceedings of the Symposium on Nonhuman Primate Models for AIDS, 1989, Abstract #60.

Roberts ED, Baskin GB, Murphey-Corb M, Fairburn B, Martin LN. Animal models for AIDS arthritis using Delta-SIVB670 in rhesus macaques. Proceedings of the Symposium on Non-Human Primate Models for AIDS, 1989, Abstract #46.

Alpers, CE, Baskin GB. Sclerosing glomerulopathy in rhesus monkeys with simian AIDS. Kid. Internat. 35:339, 1989.
(Abstract)

Baskin GB, Murphey-Corb M, and Martin L. Lentivirus-induced pneumonia in rhesus monkeys infected with SIV. Lab. Invest. 60:7A, 1989. (Abstract)

Gerber MA, Petrovic LM, and Baskin GB. Bile duct damage in acquired immunodeficiency syndrome. Hepatol 10:606, 1989.
(Abstract)

Sharer L, Epstein L, Michaels J, Murphey-Corb M, Martin L, and Baskin G. Pathology of Early Lesions in SIV Encephalitis. V International Conference on AIDS, 1989 (Abstract)

SPECIFIC AIM 2.2: Maternal-Fetal Transmission Studies

Of 12 females which were timed mated during last Fall's breeding cycle, only 2 became pregnant. These females are due late May and mid-July, respectively. Two weeks prior to term, both will be inoculated with 10 animal infectious doses of SIV/DeltaB670 and constantly monitored by video. Infants will be allowed to deliver vaginally and removed from their mothers immediately after birth before suckling to evaluate perinatal transmission.

Twenty females have been purchased and are on hand for next year's cycle. These females will be infected 2-3 months prior to estrus for evaluation of fetal infection in immunosuppressed females.

SPECIFIC AIM 2.3: Passive Protection with Monoclonal Antibodies Studies

None

SPECIFIC AIM 2.4: Rhesus Breeding for SAIDS Research

There were 346 animals in the SAIDS breeding program at the beginning of the reporting period, and 327 animals at the end of the reporting period. Twenty animals were transferred to research projects during this time period.

The Chinese rhesus population consisted of 180 animals on July 1, and 169 animals on December 31. There were 3 live births and 1 stillbirth, and 2 additional infant deaths. No adults died during the reporting period. Four animals were transferred to Dr. Michael Murphey-Corb, and 16 were transferred (sold) to Dr. Durwood Neal. The remainder were maintained for future breeding stock. This reporting period included the breeding season, and a very high proportion (over 90 percent) of the females have been palpated as pregnant.

The Indian rhesus breeding colony had 166 animals on July 1 and 158 on December 31. There were 5 live births and 1 stillbirth. There were 22 deaths: 19 adults and 4 immatures. Colitis/Amyloidosis was a major cause of death, reflecting the aging Indian colony. Nine adult animals were added to the colony in the fall, and additional funding has been requested to expand the Indian-rhesus portion of the breeding colony.

Routing care and inventory have been completed. Behavioral observations include habitat use, male social integration, and dominance and affiliative interactions of females.

Publications:

Clarke, M.R., Koritnik, D.R., Martin, L.N. and Baskin, G.B., 1989. Cage Enrichment, Physiology and Behavior in Nursery-Reared Rhesus Monkeys. Amer. J. Primatol. Supplement 1:53-58.

For the NERPRC:

SPECIFIC AIM 3a.: Characterization of the Humoral and Cellular Immune Response to SIV in terms of antibody Specifications and the Various Components of Cellular Immunity

We have defined major histocompatibility complex (MHC) class I gene products which bind certain peptide fragments of the SIV_{mac} gag protein and present those antigens to T lymphocytes. These MHC class I genes have been cloned, sequenced and expressed. We have also developed a quantitative limiting dilution assay for SIV_{mac}-specific cytolytic T lymphocytes. We have utilized this assay to demonstrate that SIV_{mac} env-specific target cell lysis is mediated by two distinct lymphocyte populations, one bearing natural killer cell-associated surface antigens and one with characteristics of a typical MHC class I restricted, antigen-specific CTL.

SPECIFIC AIM 3b.: Determination of the basis for SIV persistence

Genetic and antigenic variation may be one means by which lentiviruses that cause AIDS avoid elimination by host immune response. Genetic variation in the envelope gene (*env*) was studied by comparing the nucleotide sequences of sixteen clones obtained at 69 and 93 weeks after infection of a rhesus monkey with molecularly cloned simian immunodeficiency virus (SIV). Fifteen of these sixteen clones differed from each other and differed from the input clone over the 665 base pairs in the central region of *env* that were analyzed. Nucleotide substitutions accumulated at the rate of 6.4 per 1000 per year in this region. The majority of nucleotide substitutions (87%) resulted in amino acid changes, and variation was confined almost exclusively to three discrete segments. These results demonstrate that extensive sequence variability accumulates *in vivo* after infection with molecularly cloned virus and that selection occurs *in vivo* for changes in distinct variable regions of *env*.

SPECIFIC AIM 3c.: Determination of the pathogenesis of SIV-induced disease with emphasis on interactions of virus, origin, and cellular tropisms and host immune response

Previous experiments involving cellular tropism of SIV *in vivo* implicated tissue macrophages in lymphoid tissue as a major target cell (Ringler et al, Wyand et al, Am J Pathol 134). Subsequent experiments studying tissue macrophages *in vivo* and *in vitro* demonstrated that these cells frequently express the interleukin-2 receptor, and expression of this protein on macrophages can be predicted from measuring the soluble form of this protein in the serum. These results have just recently been published (Ringler et al, Lab Invest). Additional data involving cytokine analysis from SIV-infected alveolar macrophages and the influence of these same cytokines on SIV replication is presently being compiled.

SPECIFIC AIM 3d.: Genetic Basis for SIV Tropism

An infectious molecular clone of SIVmac(239) has been identified that induces AIDS in rhesus monkeys in a timeframe suitable for laboratory investigation. This cloned virus replicates little if at all in cultured alveolar macrophages. A variant (316) of this cloned virus was isolated from the lung of an inoculated rhesus monkey that died with AIDS. We have isolated molecular clones of 316 that confer macrophage tropism when recombined with the 239 parent. The precise molecular changes that confer macrophage tropism will be identified and the significance for disease manifestations will be determined.

SPECIFIC AIM 3e.: Evaluation of an approach to SIV vaccine development using a defective virus

A 3.5kb SacI fragment encompassing env gene sequences of cloned SIVmac239 DNA was subcloned into the retrovirus vector pZipneoSV(X). A 5.6 kb NarI-SphI fragment of cloned SIVmac239 DNA encompassing gag, pol, vif, vpx, and vpr was also subcloned into the same retrovirus vector. The orientations of the inserts were identified by restriction endonuclease mapping. The sense orientation constructs were designated SIVgp⁺ (gag-pol containing) and SIVenv⁺ (env containing). Similarly, antisense constructs were designated SIVgp⁻ and SIVenv⁻. All constructs were transfected into the amphotropic packaging cell line GPenVAM12 (kindly provided by Dr. Arthur Bank of Columbia University). The packaging cell line provided all the necessary viral proteins to encapsidate retroviral RNA. High titer virus stocks were harvested ($10^5 - 10^6$ cfu/ml). Four animals were inoculated with sense construct recombinant virus (5×10^5 cfu) and antibody response to the recombinant virus infection was monitored by ELISA.

		<u>Day 0</u>	<u>Wk2</u>	<u>Wk4</u>	<u>Wk6</u>	<u>Wk8</u>	<u>Wk10</u>	<u>Wk12</u>	<u>Wk15</u>
SIVgp ⁺	Mm181-88	0.023	0.054	0.106	0.051	0.117	0.077	0.066	0.025
	Mm230-88	0.010	0.023	0.044	0.038	0.095	0.077	0.152	0.056
SIVenv ⁺	Mm277-88	0.080	0.211	0.126	0.095	0.130	0.083	0.075	0.040
	Mm356-88	0.013	0.344	0.465	0.474	0.573	0.198	0.188	0.119

These results suggest that Mm 356-88 may have produced anti-SIV antibodies in response to exposure to the SIVenv⁺ retrovirus vector. These results will need to be confirmed by other specific tests.

The ability of antisense constructs to inhibit SIV replication in tissue culture was investigated. Gpenvam12 cells transfected with antisense constructs were co-cultivated with HUT-78 cells. Unc cloned SIVmac virus was used to infect those antisense transduced HUT-78 cells. Virus replication was monitored by assay of reverse transcriptase (RT) activity in the culture supernatant. Both SIVgp⁻ and SIVenv⁻ antisense

transduced HUT-78 cells yielded background RT activity whereas parental or sense construct (SIVgp⁺ and SIVenv⁺) transduced HUT-78 cells revealed at least 25 fold higher RT activity. These results suggest that antisense RNA expression can inhibit SIV replication in culture by at least 96 percent.

SPECIFIC AIM 3g.: Development of infectivity assays for SIV by the intravenous, oral, vaginal, and other routes

Preliminary data described in the last progress report failed to implicate the passage of SIV through intact squamous mucosa. We have documented that SIV can traverse across abraded squamous mucosal surfaces. Because of the relatively good barrier of intact mucosa for SIV and the similarity of SIV infection through abraded mucosa to direct inoculation intravascularly, we have chosen instead to concentrate our efforts on disease pathogenesis.

For the YRPRC:

SPECIFIC AIM 3a.: Monitoring of Chronically Infected Macaques

We have continued to monitor a group of macaques chronically infected with SIV_{smm}; 8 of 13 animals (61.5%) died from an AIDS-like disease between 14 and 42 months post-infection. The remaining 5 animals have been monitored for periods of 42 months (2 animals) to 55 months (3 animals). One of the latter animals is showing immunosuppression, lymphadenopathy and splenomegaly; the other four animals continue to appear clinically normal. Sentinel animals, housed in individual cages in the same room with infected animals (5 animals), or in the same cage with an infected animal (4 sexually immature animals), continue to be seronegative (by conventional methods) and virus negative after 34 to 55 months. However, recent preliminary studies of two of the cage contacts by PCR and the PWM technique (see below) suggest that both of these animals are virus infected. Studies are underway to confirm these preliminary observations.

SPECIFIC AIM 3b.: Isolation of SIV from Stumptail Macaques

As noted previously, widespread lentivirus infection has been documented in a stumptail macaque colony at the Yerkes Center (by serology and virus isolation). This colony has continued to show a high morbidity and high mortality rate, with 22 of 32 animals dying since mid-1988. Major findings at necropsy in these animals include severe weight loss and opportunistic infections (candidiasis and mycobacteriosis). Animals checked have shown significant reductions in lymphocyte counts, reduced number of CD4⁺ cells and decreased CD4⁺/CD8⁺ cell ratios. Efforts are currently underway in collaboration with Drs. P. Johnson and V. Hirsch, Georgetown University, to clone and sequence this isolate for comparison with other SIV isolates. Studies will be initiated in the near future

to evaluate the pathogenicity of this isolate (SIV_{stm}) for other macaque species.

SPECIFIC AIM 3c.: SIV_{smm} and STLV-1 Infection in the Yerkes Mangabey Colony

Efforts are continuing to establish and monitor the SIV_{smm} and STLV-1 infection status of the Yerkes mangabey breeding colony. We continue to see a correlation between age and seropositivity for SIV, with more than 90% of animals that are 9 years of age or older being seropositive. These determinations will be used to determine the incidence of neonatal infection, presence of maternal antibodies in infants, age of seroconversion and virus infection, to provide information on the epidemiology and modes of transmission within the colony, and to establish a retrovirus-free mangabey breeding colony.

Attempts to establish a retrovirus-free mangabey breeding colony have been initiated by removal of 13 seronegative mangabeys from the main breeding colony (one adult male, 7 sexually mature females and 5 sexually immature animals). These animals will be individually caged and retested at monthly intervals to confirm their virus negative status. Following three successive negative tests, the subjects will be formed into a single social group and managed to promote optimal breeding. Following group formation, the subjects will continue to be monitored at quarterly intervals, and all newborns will be tested during the first month of life. The long-range goal of this effort is to insure the availability of virus-free mangabeys for studies related to the AIDS Animal Model Program by maintaining and expanding a viable breeding unit of seronegative mangabeys.

SPECIFIC AIM 3d.: Perinatal Transmission of SIV_{smm}

Studies to evaluate the perinatal transmission of SIV_{smm} were initiated by infecting 15 timed pregnant rhesus macaques with SIV_{smm} during various stages of gestation and monitoring the offspring for evidence of virus infection. Three groups of five animals were experimentally infected with SIV_{smm} during early (day 28 to 35), mid (day 78-78), and late (day 146-150) gestation. Offspring delivered by these experimentally infected macaques included 2 stillbirths and 13 live births; 1 of the latter died at 3 days of age. There was no direct evidence that the stillbirths or neonatal deaths were related to SIV infection and the other 12 infants (PBMC) and milk samples from the mothers were negative following virus culture at 1 week of age or less and at 3 months and 6 months after birth. However, studies utilizing PCR and the PWM assay indicated that at least 2 and possibly 4 of the 12 infants were SIV infected. One of the 12 infants has subsequently become virus positive following culture of the PBMC at 9 months after birth. This study indicates that mother to infant transmission of SIV does occur in the macaque model. However, since these infants were allowed to remain with the mother for the first year of life, it is not known at present whether transmission occurred in utero or via ingestion

of milk from SIV-infected mothers. These data are being further analyzed to see if the mode of transmission can be more definitively determined.

One SIV-infected adult female died at 15 months post-infection. The other infected females will be used in a continuing breeding program to determine if pregnancy in animals that are SIV infected at the time of conception results in a higher incidence of perinatal transmission of SIV. This continuing breeding program will also be designed to allow an assessment of sexual transmission of SIVs (male to female and female to male).

SPECIFIC AIM 3e.: Retrovirus Seroprevalence in Feral African Monkeys and Isolation of a Lentivirus from African Sykes Monkeys

As noted previously, serologic surveys of African green monkeys, baboons and Sykes monkeys in Kenya revealed a high seropositive rate for antibodies to SIV and STLV-1 in African green and Sykes monkeys. We subsequently received six SIV-seropositive and 3 seronegative Sykes monkeys from the Institute of Primate Research in Kenya for use in attempts to isolate an SIV from this species.

As a result of these studies, a lentivirus has been isolated from five of six seropositive Sykes monkeys and efforts are currently underway to characterize the *in vitro* properties of this isolate. Efforts are also underway, in collaboration with Drs. Johnson and Hirsch of Georgetown University to clone and sequence this isolate for comparison with other SIV isolates. Studies are also planned to evaluate the pathogenicity of this isolate in rhesus and pig-tailed macaques.

SPECIFIC AIM 3f.: Determination of SIV Infection in Mangabeys by In Vitro Polyclonal Activation of Peripheral Blood Mononuclear Cells by Pokeweed Mitogen (PWM Assay) and by PCR

A technique recently developed in our laboratory to determine the presence of antibodies to SIV entails the culture of PBMC with pokeweed mitogen (PWM) and subsequent assay of supernatant fluids for SIV antibody. In preliminary studies utilizing this technique, PBMC from seropositive and seronegative (by ELISA, RIPA, and Western blot) sooty mangabeys and rhesus macaques were analyzed. As expected, supernatant fluids from PBMC cultures of seronegative rhesus were negative for SIV antibodies and supernatant fluids from PBMC cultures of seronegative mangabeys and rhesus were positive for antibodies to SIV. Surprisingly, supernatant fluids from PBMC cultures of seronegative mangabeys had significant antibody titers to SIV. In addition, depletion of CD8⁺ T cells from the PBMC from seronegative mangabeys prior to culture with PWM resulted in marked increases in titers of SIV antibodies. Preliminary studies with PCR using *gag* sequence primer pairs have shown that these "seronegative" mangabeys are,

in fact, infected with SIV. Studies are continuing to confirm these observations and to sequentially monitor such mangabeys to determine if and when they become seropositive by conventional techniques and to evaluate the circumstances surrounding such seroconversion.

SPECIFIC AIM 3g.: SIV-Induced Disease in Naturally Infected Mangabey Monkeys

Although it is generally believed that natural SIV infection in African species of non-human primates (e.g., African green monkeys and mangabeys) does not result in clinical disease, we have noted occasional disease problems (e.g., CMV, Noma, amebiasis) in mangabeys that might have been associated with an immunosuppressive SIV infection. More recently, two adult mangabeys that died had histologic lesions characteristic of those seen in macaques experimentally infected with SIV. The first case, a 23-year old female, wildborn mangabey, was found to have diffuse lymphoid infiltrates in the lungs (comparable to lymphoid interstitial pneumonia) with numerous syncytial giant cells throughout the lungs. This animal had a clinical history of chronic lymphocytosis of seven years duration, with white blood cell counts ranging between 22,700 and 31,100 during that period. The second case occurred in a 16-year old colony born mangabey that developed T-cell leukemia during her last 2 years of life. During this time, the animal's white blood cell count increased to 127,500, with 94% lymphocytes (98% of these were T cells, 87% of which were CD8⁺ cells). This animal was positive for both SIV and STLV-1, and blood transfusion studies were done to determine if T cell leukemia could be transmitted to STLV-1 and SIV-negative mangabeys, rhesus or pig-tailed macaques (2 recipients of each species). The mangabey recipients are currently clinically normal (6 months post-transfusion), the rhesus macaques are showing immunosuppression and both pig-tailed macaques died within 5 months of the transfusion. These animals showed diarrhea and weight loss, severe progressive immunosuppression and clinical evidence of CNS disease. Histologically, lesions were limited primarily to the brain and spinal cord and consisted of a severe, granulomatous meningoencephalomyelitis that contained large multinucleated giant cells.

These observations indicate that SIV, on occasion, may be pathogenic in mangabeys, and that the isolate (or dual STLV-1 and SIV infection) from the mangabey with T cell leukemia was especially neurotrophic when given to pig-tailed macaques. Studies are currently underway in collaboration with Dr. J. Ribas, AFIP, to determine if SIV antigens can be demonstrated in the multinucleated giant cells (by immunohistochemistry) of mangabeys with apparent SIV-induced disease.

SPECIFIC AIM 3h.: Mangabey and Rhesus and Pig-tailed Macaque Breeding Colonies

At the present time, there are 80 rhesus monkeys, 130 mangabey monkeys and 92 pig-tailed macaques contributing to the expanded breeding and related objectives of this project, and plans are to acquire an additional 50 pig-tailed macaques as soon as possible. The rhesus and mangabey breeding colonies continue to reproduce very successfully; the productivity of the pig-tailed macaque breeding colony has been somewhat less successful. The productivity of these breeding colonies for the calendar year 1989 is summarized in the following table:

Summary of AIDS Animal Model Breeding Colonies
January 1, 1989 through December 31, 1989

	<u>Jan.-June</u>	<u>July-Dec.</u>	<u>Totals</u>
<u>Mangabey</u>			
Total Births	22	6	28
Stillborn/Neonatal Loss	5	1	6
Surviving	17	5	22
<u>Pigtail</u>			
Total Births	11	7	18
Stillborn/Neonatal Loss	5	3	8
Surviving	6	4	10
<u>Rhesus</u>			
Total Births	51	5	56
Stillborn/Neonatal Loss	5	1	6
Surviving	46	4	50

4. Plans for Next Six Months

During the next six months, studies described above will be continued and expanded as appropriate.

5. Publications (July 1, 1989 - December 31, 1989)

Powell, J.D., McClure, H.M., Anderson, D., Fultz, P.N., Sell, K.W., Ahmed-Ansari, A.: Phenotypic and functional differences in NK and LAK cells in the peripheral blood of sooty mangabeys and rhesus macaques. *Cell Immunol.* 124:107-118 (In press).

McClure, H., Anderson, F., Fultz, P., and Ansari, A.: Non-Human primate models for AIDS research. *International Symposium on Biomedical Research Strategy on AIDS*, Yverdon-les-Bains, Switzerland, September 24-28, 1989 (Abstract)

Villinger, F., Jehuda-Cohen, T., Powell, J.D., Anderson, D., McClure, H.M., and Ansari, A.A.: Relative differences in the regulation and susceptibility for the replication of SIV/SMM in CD4⁺ T cells and monocytes from asymptomatic mangabeys and disease susceptible rhesus macaques. *Seventh Annual Symposium on Non-Human Primate Models for AIDS*, Portland, Oregon, October 11-13, 1989, p.42 (Abstract)

McClure, H.M., Anderson, D.C., Brodie, A., Lockwood, E., Fultz, P., Ansari, A., and Gordon, T.: T-cell leukemia in mangabey monkeys from a colony with a high incidence of SIV and STLV-1 infection. *Seventh Annual Symposium on Non-Human Primate Models for AIDS*, Portland, Oregon, October 11-13, 1989, p. 61 (Abstract).

Ansari, A.A., Powell, J.C., Jehuda-Cohen, T., Villinger, F., Anderson, D., and McClure, H.M.: Delineation of peptide specific T cell immunity in SIV/SMM infected non-human primates using the cellular western blot assay. *Seventh Annual Symposium on Non-Human Primate Models for AIDS*, Portland, Oregon, October 11-13, 1989, p. 70 (Abstract).

Powell, J.D., Jehuda-Cohen, T., Villinger, F., Anderson, D., McClure, H.M., and Ansari, A.A.: CD8⁺ T cells from SIV seronegative sooty mangabeys suppress SIV/SMM virus replication in vitro. *Seventh Annual Symposium on Non-Human Primate Models for AIDS*, Portland, Oregon, October 11-13, 1989, p. 72 (Abstract).

Jehuda-Cohen, T., Slade, B., Powell, J.D., Villinger, F., Anderson, D., McClure, H.M., Sells, K.W., and Ansari, A.A.: A novel method for the detection of antibodies against SIV and HIV in conventionally seronegative sooty mangabeys and high risk humans. *Seventh Annual Symposium on Non-Human Primate Models for AIDS*, Portland, Oregon, October 11-13, 1989, p. 76 (Abstract).

Fultz, P.N., Stricker, R.B., McClure, H.M., Anderson, D.C., Switzer, W., and Horaist, C.: The role of antibodies in protection from or potentiation of disease in SIV/SMM-infected mangabeys and macaques. International TNO Meeting on Animal Models in AIDS, Maastricht, the Netherlands, October 23-26, 1989, p. 20-21 (Abstract).

Ribas, J.L., Schlessinger, R., Anderson, D.C., McClure, H.M., and Mirra, S.: Immunocytochemical and histopathological study of meningoencephalitis in macaque monkeys induced by simian immunodeficiency virus. International TNO Meeting on Animal Models in AIDS, Maastricht, The Netherlands, October 23-26, 1989, p. 46 (Abstract).

McClure, H.M., Anderson, D.C., Fultz, P., Ansari, A., and Schinazi, R.: Non-Human primate models for AIDS therapy. Second International Conference on Drug Research in Immunologic and Infectious Diseases: Acquired Immune Deficiency Syndrome (AIDS), Arlington, Virginia, November 6-9, 1989, Abstract #24 (Abstract).

Fultz, P.N., Anderson, D.C., McClure, H.M., Sonigo, P., Montagnier, L., and Stricker, R.B.: Virulence and Variation of Viruses: Coalition with Cells, Cytokines and Simians. AIDS Res. Human Retroviruses 6:70 (1990) (Abstract) (In press).

Fultz, P.N., Stricker, R.B., McClure, H.M., Anderson, D.C., Switzer, W.M., and Horaist, E.: Humoral Response to SIV/SMM Infection in Macaque and Mangabey Monkeys. J. Acquired Immune Deficiency Syndromes 3:319-329 (1990) (In press).

McClure, H., Anderson, D., Gordon, T., Ansari, A., Fultz, P., Klumpp, S., Emau, P., and Isahakia, M.: Natural SIV Infections in Non-Human Primates. XIII International Primatological Society Meeting (In press).

Fultz, P.N., Gordon, T.P., Anderson, D.C., and McClure, H.M.: Prevalence of Natural Infection with SIV_{SMM} and STLV-1 in a Breeding Colony of Sooty Mangabey Monkeys. AIDS Res. Human Retroviruses (In press).

Gust, D.A., Busse, C.D., and Gordon, T.P.: Reproductive Parameters in the Sooty Mangabey (Cercocebus torquatus atys). Am. J. Primatol. (In press).

Gust, D.A. and Gordon, T.P.: Male Age and Reproductive Behavior in Sooty Mangabeys (Cercocebus torquatus atys). Animal Behavior (In press).